The Photochemistry of Phosphorus Compounds. Part X.[1] Photolysis of Disodium α-D-Glucose 6-Phosphate in Aqueous Solution under Nitrogen or Oxygen

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The reactions of disodium α-D-glucose 6-phosphate in aqueous solutions upon irradiation at 254 nm in the presence of either nitrogen or oxygen were investigated, using g.l.c.–mass spectrometry, paper chromatography, paper electrophoresis, colorimetric tests, and kinetic methods for identification of products. The main photolysis products were 6-phosphogluconate, arabinose 5-phosphate, phosphoglycerate, phosphoglycolate, carbon dioxide, and orthophosphate. Glucose was not formed. Under oxygenative conditions only, an acid-labile phosphate ester was also formed, possibly a triose phosphate. The photolysis of glucose 6-phosphate caused the appearance of an u.v. absorbing band at 275 nm, presumably due to production of an αβ-unsaturated carbonyl compound. This band was formed in high yield by irradiation under nitrogen, but was weak in oxygenated solutions. The photolysis of trisodium 6-phosphogluconate was shown to yield an arabinose phosphate, thus indicating that 6-phosphogluconate may be an intermediate in the photolytic conversion of glucose 6-phosphate into arabinose 5-phosphate.

GLUCOSE 6-PHOSPHATE (G-6-P) in aqueous solution was shown [2] to have a weak, shallow absorption band in the 280—240 nm region, attributed to an internal electronic transition, and strongly rising absorption below 220 nm, assigned to a ' charge transfer to solvent ' transition.[1-5] The present work extends our previous studies on the photochemistry of inorganic phosphates,[3] ethyl dihydrogen phosphate,[4] and glycerol 1- and 2-phosphate [5] to D-glucose 6-phosphate. In spite of the considerable importance of this sugar phosphate as a key intermediate in the glycolytic pathway of carbohydrate metabolism, only limited data have been reported on its ' photochemical hydrolysis,' and the products of photolysis were not identified.[6] The only related studies have been on its radiolytic decomposition by ionizing radiation,[7] on its hydrolysis, and on other ' dark ' reactions in acid and alkaline solution.[8] Also, a quantum chemical study has been made on the charge distribution in the glucose 6-phosphate molecule ion.[9] On the other hand, the photolysis [10] and radiolysis [11] of D-glucose and of other sugars in water solutions have been carefully investigated. These studies do not permit a prediction of the chemical consequences of electronic excitation of glucose 6-phosphate. In the present work, we chose to irradiate glucose 6-phosphate at 254 nm, a region in which the photolytic decomposition of water is negligible, and in which therefore the effect of light is directly on the sugar phosphate molecule ion.

EXPERIMENTAL

Materials.—Disodium D-glucose 6-phosphate, trisodium 6-phosphogluconic acid (both Sigma) and [^{14}C]-D-glucose 6-phosphate (uniformly labelled; International Chemical and Nuclear Corp.) were used without further purification. Water used for the photolysis was twice distilled.

Irradiations.—Procedures for irradiation were as previously described [1] using a medium pressure mercury lamp (Hanau, Q-81 or TQ 150) fitted into a double-jacketted quartz photochemical reactor. Distilled water thermostatted at 30° was circulated between the lamp and the reaction solution, to assure a constant temperature and also to filter out the far u.v. (185 nm) emission of the lamp. Thus, most of the light absorbed in the solution was due to the 254 nm line of the mercury arc. The irradiated sugar phosphate solution (usually 0·01M) had an optical path of *ca.* 1 cm, and a volume of *ca.* 150 ml. It was stirred by a gas stream (either nitrogen or oxygen) issuing from a sintered glass disk at the bottom of the reaction vessel. Samples were withdrawn by a syringe pierced through a rubber septum fitted in a side port of the reactor.

For photolysis of solutions of either [^{14}C]glucose 6-phosphate or 6-phosphogluconate, a smaller double-jacketted reactor was used, consisting of an inner quartz tube which was charged with 2 ml of reactant solution, and through which either nitrogen or oxygen was bubbled. Distilled water at 30° was pumped through the outer jacket, and the whole assembly was placed next to a low-pressure mercury lamp (Thermal Syndicate model T/M5/544).

Analysis of Photolysis Products.—In order to facilitate comparison, the various products were determined simultaneously during the course of the reaction.

Carbon dioxide. The gas issuing from the reactor was passed *via* a three-way stopcock through a train of two barium hydroxide absorption flasks (100 ml; 0·02M) in series. After an interval, the stopcock was turned to lead the gas stream into an alternative train of two barium hydroxide absorption flasks, while portions of the first two flasks were titrated with hydrochloric acid (0·1N; phenolphthalein). The gas stream was then switched to a third

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[3] M. Halmann and I. Platzner (*a*) *Proc. Chem. Soc.*, 1964, 261; (*b*) *J. Chem. Soc.*, 1965, 1440; (*c*) *J. Phys. Chem.*, 1966, **70**, 2281; (*d*) Ch. Benderli and M. Halmann, *ibid.*, 1967, **71**, 1053.
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[5] J. Greenwald and M. Halmann, *J.C.S. Perkin II*, 1972, 1095.
[6] H. Trapmann and M. Devani, *Naturwissenschaften*, 1965, **52**, 208.
[7] N. K. Kochetkov, L. I. Kudryashov, M. A. Chlenov, and L. P. Grineva, (*a*) *Zhur. obschchei Khim.*, 1971, **41**, 2071; (*b*) *Doklady Akad. Nauk S.S.S.R.*, 1972, **202**, 847.
[8] Ch. Degani and M. Halmann, *J. Amer. Chem. Soc.*, 1966, **88**, 4075; (*b*) C. A. Bunton and H. Chaimovich, *ibid.*, p. 4084.
[9] R. Gaspar, jun., *Acta Biocheim. Biophys. Acad. Sci. Hung.* 1972, **7**, 275, 285.
[10] (*a*) T. C. Laurent, *J. Amer. Chem. Soc.*, 1956, **98**, 1875; (*b*) G. O. Phillips and G. J. Moody, *J. Chem. Soc.*, 1960, 3398; (*c*) G. O. Phillips and T. Rickards, *ibid.*, 1969, 455.
[11] (*a*) G. O. Phillips, G. J. Moody, and G. L. Mattock, *J. Chem. Soc.*, 1958, 3522; (*b*) V. Hartmann, C. V. Sonntag, and D. Schulte-Frohlinde, *Z. Naturforsch.*, 1970, **25b**, 1394; (*c*) J. Schubert and E. B. Sanders, *Nature New Biology*, 1971, **233**, 199.

train of absorption flasks, while the second train was sampled for titration, *etc.* (see Table 1).

Orthophosphate. Orthophosphate produced by the photolysis was determined for samples (5 ml) using the method of Fiske and Subba Row.[12a]

Glucose 6-phosphate. This was determined enzymically by the reduction of NADP in the presence of glucose 6-phosphate dehydrogenase.[12b]

Acid formation. Any acid formed during the photolysis was determined as the difference in the titration value by 0·1N-sodium hydroxide to the phenolphthalein end-point, before and after photolysis (see Table 2).

Acid-labile phosphate. In order to facilitate comparison with Pontis and Leloir's tabulated data on rates of acid-catalysed hydrolysis of various sugar phosphates,[13] the reaction was carried out in N-sulphuric acid at 100°. To the photolysed solution (2 ml) sulphuric acid (10 ml; 5N) was added and the mixture was diluted with water to 50 ml. The flask was placed in a water-bath at 100° and portions (5 ml) were withdrawn at various intervals for orthophosphate analysis by the method of Fiske and Subba Row. In parallel, the rate of hydrolysis of a solution of non-photolysed glucose 6-phosphate was measured in N-sulphuric acid. The difference in release of orthophosphate between the two runs (without and with photolysis) was then plotted semi-logarithmically against time, to obtain the kinetic curve for the acid-catalysed hydrolysis of an acid-labile phosphate.[14]

Colorimetry.—Tests for potential photolysis products were made with the following reagents: [15] 2,7-dihydroxynaphthalene ($\lambda_{max.}$ 550 and 680 nm; test for formaldehyde, glycolate, lactate); chromotropic acid (570 nm; formaldehyde glycolate, arabinose); orcinol (670 nm; pentoses, tetroses); cysteine–sulphuric acid (415 nm; pentoses). Results were in all tests negative or weak after photolysis under nitrogen, but strongly positive after photolysis under oxygen.

Paper Electrophoresis and Paper Chromatography.—These were carried out as previously described.[1] Phosphorus compounds on the paper were detected with a molybdate spray,[16a] while sugars and other reducing substances were detected with aniline hydrogen phthalate,[16b] silver nitrate,[16a] or triphenyltetrazolium chloride.[16d] The sensitivity of detection methods enabled identification of 1—2% yields of photolysis products, starting from 0·01M-glucose 6-phosphate.

For the ^{14}C-labelled products, the chromatograms were cut into 1 × 2·5 cm^2 strips for counting by liquid scintillation.

Of the various solvents used (see Tables 3 and 4), best results were obtained with solvents A, B, and particularly C and F.

Following photolysis under nitrogen, one of the products, identified as a sugar by its positive colour reaction with silver nitrate, was found to migrate approximately as glucose in all solvents except A. This proved that glucose was *not* a product of the photolysis of glucose 6-phosphate. The unknown compound, after separation by elution from an anion exchange resin (Dowex 1) was shown to be neither glucohexodialdose [7] (by reduction with sodium borohydride and search for sorbitol) in solvent C, nor 1,6-anhydroglucopyranose [17] (by chromatography in solvent F).

During photolysis of glucose 6-phosphate under oxygen, two other phosphorus-free products were detected by paper chromatography (solvent C with $R_{G\text{-}6\text{-}P}$ 5·8—6·0 and 18), using silver nitrate spray as the detector, but they could not be identified as any of the sugars or acids tested (as listed in Table 3).

The direct separation of phosphate esters by unidimensional paper chromatography is difficult.[13] Excellent separation was, however, achieved by paper chromatography of the free sugars, released after enzymic hydrolysis of the end group of phosphate esters. Results obtained after the application of alkaline phosphatase on the products of photolysis of glucose 6-phosphate under nitrogen and oxygen, respectively, are presented in Table 4. Chromatography in solvent C of the hydrolysis products of photolysis under nitrogen revealed the formation of four sugars, in addition to glucose, with $R_{G\text{-}6\text{-}P}$ 5·3, 5·8, 10·8, and 13·5. Of these, only the product with $R_{G\text{-}6\text{-}P}$ 10·8 could be assigned as being either fructose or arabinose. This product was more definitely identified as being only arabinose by paper chromatography in solvent F. It must, therefore, be concluded that arabinose 5-phosphate is one of the products of the photolysis of glucose 6-phosphate under nitrogen. By the same methods, arabinose 5-phosphate was also shown to be an important product of the photolysis of glucose 6-phosphate under oxygen. Under oxygenated conditions, another spot, $R_{G\text{-}6\text{-}P}$ 7, could be identified as being due to gluconic acid. Hence, the original photolysis product, before enzymic hydrolysis, must have been 6-phosphogluconic acid.

G.l.c. and Mass Spectrometry.—Silylation of sugar phosphates was carried out [18] by adding to 0·5—1 mg of the dried residue remaining after lyophilization of the photolysis reaction mixture (or to known sugar phosphates as reference compounds) pyridine (30 μl), BSTFA [*NO*-bis(trimethylsilyl)trifluoroacetamide] (50 μl), and TMCS (trimethylchlorosilane, both from Pierce Chemical Co.) (20 μl). After 2—3 h at room temperature, the solution was injected into a gas chromatograph–mass spectrometer (LKB Producter, model 9000), using SE30 (3%) on Gaschrom Q (AW-DMCS), with a helium flow rate of 30 ml min^{-1}, injection temperature 250°, column temperature programmed from 100 to 230° after 2 min delay at 100° (5° min^{-1}), mass spectrometer ion source 70 eV, 50 μA. The photolysis products were identified by comparison of the observed retention times and mass spectral patterns (Table 5) of the chromatographic peaks with those of the reference compounds, and also with reported data.[18] The assignment of

[12] L. F. Leloir and C. E. Cardini, in ' Methods in Enzymology,' eds. S. P. Colowick and N. O. Kaplan, Academic Press, New York, 1957, vol. III (*a*) p. 843; (*b*) p. 153.

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[16] I. M. Hais and K. Macek, ' Paper Chromatography,' Publishing House of the Czechoslovak Academy of Science, Prague, 1963 (*a*) p. 819; (*b*) p. 793; (*c*) p. 782; (*d*) p. 783.

[17] E. J. Bourne, G. M. Lees, and H. Weigel, *J. Chromatog.*, 1963, **11**, 253.

[18] (*a*) J. A. McCloskey, A. M. Lawson, K. Tsuboyama, P. M. Krueger, and R. N. Stillwell, *J. Amer. Chem. Soc.*, 1968, **90**, 4182; (*b*) M. Zinbo and W. R. Sherman, *ibid.*, 1970, **92**, 2105; (*c*) F. Eisenberg, jun., and A. H. Bolden, *Analyt. Biochem.*, 1969, **29**, 284; (*d*) F. Eisenberg, jun., in 'Analytical Chemistry of Phosphorus Compounds,' ed. M. Halmann, Wiley–Interscience, New York, 1972, pp. 81—83; (*e*) W. W. Wells, T. Katagi, R. Bentley, and C. C. Sweeley, *Biochim. Biophys. Acta*, 1964, **82**, 408; (*f*) G. H. Lorimer, T. J. Andrews, and N. E. Tolbert, *Biochemistry*, 1973, **12**, 18.

the α- and β-anomers of the sugar phosphates, which were in most cases well separated, was made by analogy with that for the corresponding sugars as in previous reports.[18c,e] The observed retention times, relative to β-glucose 6-phosphate, for the trimethylsilyl derivatives tested, are as follows: orthophosphate 0·19; phosphoglycolate 0·38; phosphoglycerate 0·57; α-arabinose 5-phosphate 0·79; β-arabinose 5-phosphate 0·82; α-ribose 5-phosphate 0·80; β-ribose 5-phosphate 0·81; α-mannose 5-phosphate 0·92; β-mannose 5-phosphate 0·97; α-glucose 6-phosphate 0·98; 6-phosphogluconate 1·03.

After photolysis under oxygen, the products clearly identified were orthophosphate, phosphoglycolate, phosphoglycerate, α- and β-arabinose 5-phosphates, α- and β-glucose 6-phosphates, and 6-phosphogluconate. After photolysis under nitrogen only the α- and β-anomers of arabinose 5-phosphate and of glucose 6-phosphate were detected.

RESULTS

Kinetic Studies.—(a) *Photolysis under nitrogen.* If the primary degradation process of glucose 6-phosphate under u.v. irradiation were the release of orthophosphate, the rate of disappearance of the sugar phosphate (which we measured enzymically with glucose 6-phosphate dehydrogenase) should be equal to the rate of appearance of orthophosphate. Unfortunately, the experimental error in the enzymic assay (*ca.* 2%) was as large as the observed disappearance of glucose 6-phosphate during the period of irradiation (≤24 h), and the expected equality with the release of orthophosphate could not be checked directly. However, the considerable quantities of carbon dioxide simultaneously evolved (see Table 1), which were approximately double the

TABLE 1

Disappearance of glucose 6-phosphate and release of orthophosphate and of carbon dioxide during the photolysis of glucose 6-phosphate under O_2 and N_2

	Photolysis under O_2			Photolysis under N_2		
	CO_2	HPO_4^{2-}	G-6-P	CO_2	HPO_4^{2-}	G-6-P
t/h	(%)	(%)	(%)	(%)	(%)	(%)
0	0	0·25	100	0	0·7	100
1	0·38			0·85	1	98
1·5		1·5	99			
2	1·53	2	93	1·7	1·25	98
3	3·05			2·8		
3·1		3·5	93·5			
4	4·6			3·8	1·75	99
4·1		5·05	83			
6	8·9	8	81·5	5·1	2·25	96
8	15·4	12·5	73	6·4	2·75	89
10	21·5	16·8	75	7·7	3·25	97
24	70	37	23·8	10·8	5·5	97

amounts of orthophosphate liberated, prove that the reaction is *not* a simple release of orthophosphate. The more rapid release of carbon dioxide relative to orthophosphate proves the formation of some other sugar phosphate. The absence of acid formation during photolysis under nitrogen (Table 2) also suggests that glucose 6-phosphate undergoes degradation to other sugar phosphates, of shorter carbon chain lengths, as well as to some neutral non-phosphorus-containing products.

(b) *Photolysis under oxygen.* In the presence of oxygen, the decomposition of glucose 6-phosphate was quite rapid. As Table 1 shows, the rate of release of orthophosphate was about five times faster than under nitrogen. The yield of carboxylic acids produced under these conditions was substantial (see Table 2), thus indicating that oxidation had occurred during the photolysis. The rate of release of orthophosphate was slower than that of the disappearance of glucose 6-phosphate (Table 1), which proves that some other organic phosphates were formed (which did not react with

TABLE 2

Acid formation during the photolysis of glucose 6-phosphate under O_2 and N_2

Run no.	Atmosphere	Irradiation time (h)	Acids formed (mole %)	HPO_4^{2-} formed (mole %)
1	O_2	2·5	45·5	18·5
6 *	O_2	2·5	29·2	12
8	O_2	24	55	30
4	N_2	3	Trace	6·5
5	N_2	2·5	Trace	5·5

* The difference in results between runs 1 and 6 is due to a change in the relationship of the irradiated solution to the light source.

glucose 6-phosphate dehydrogenase). The rate of production of carbon dioxide was initially (during the first 5 h, see Figure 1) equal to that of the release of orthophosphate, but

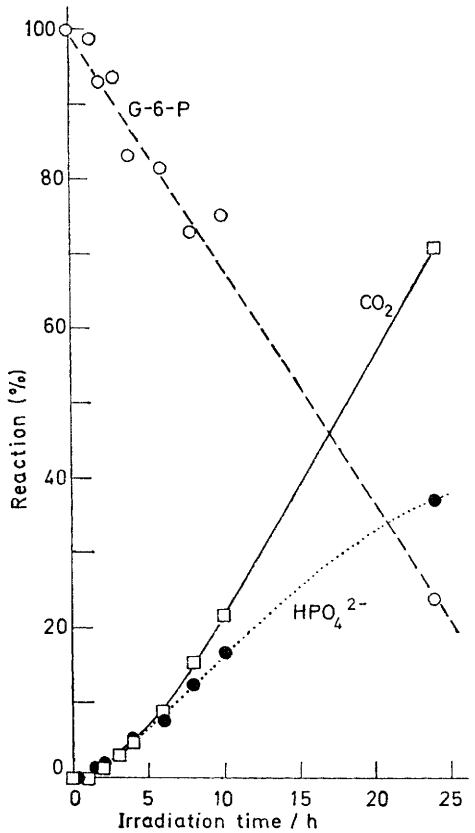


FIGURE 1 Photolysis of disodium D-glucose 6-phosphate (0·01M) in oxygenated solution. Formation of carbon dioxide and orthophosphate as a function of time

increased more rapidly during the later stages of photolysis, presumably owing to secondary reactions.

Acid-labile Phosphates.—Results of the test for acid-labile phosphate esters, those undergoing relatively rapid hydrolysis in 0·5M-sulphuric acid at 100°, are presented in Figure 2. A complication in this search is the acid-catalysed hydrolysis of glucose 6-phosphate itself (which is not negligible under these conditions, having a half-life $t_{\frac{1}{2}}$ 22—24 h),[13] and which we therefore had to subtract from the total orthophosphate

released by acid hydrolysis of the photolysed solutions (see Experimental section). As shown in Figure 3, during photolysis under nitrogen, no appreciable amount of acid-labile (*i.e.* much more acid-labile than glucose 6-phosphate) phosphate ester was produced. However, photolysis under oxygen (bottom curve of Figure 2) caused marked production of such an acid-labile phosphate. From a semi-log plot

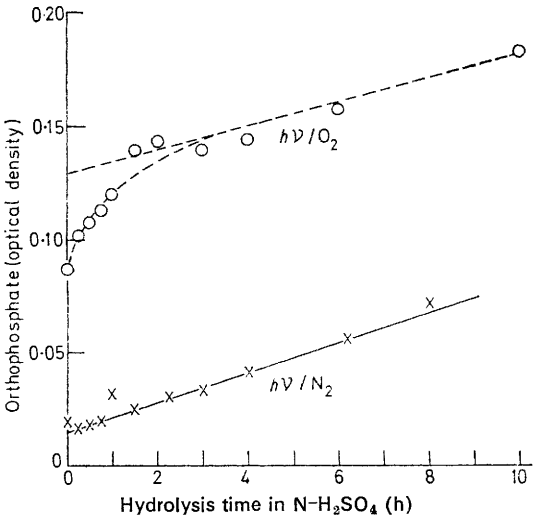


FIGURE 2 Acid-catalysed hydrolysis (in N-H_2SO_4 at 100°) of the photolysis mixture, after irradiation for 6 h under oxygen or 10 h under nitrogen

of the decay of this acid-labile phosphate, the half-life for hydrolysis was obtained, $t_{\frac{1}{2}}$ 30 min. This is similar to the values reported for triose or tetrose phosphates,[14] *e.g.* 33 min for dihydroxyacetone phosphate.

U.v. Spectra.—The photolysis of aqueous solutions of glucose 6-phosphate resulted in formation of a product absorbing in the 265—270 nm region. This absorption was weak after irradiation under oxygen but was very intense following irradiation under nitrogen. The evolution of the absorption maximum at 270 nm as a function of time, for

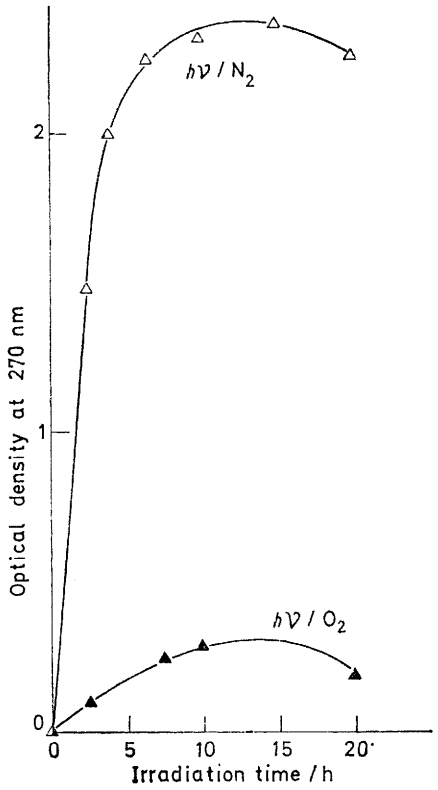


FIGURE 3 Development of the 270 nm absorbing band during the photolysis of glucose 6-phosphate (0·01M) under oxygen and nitrogen. Optical path 1·0 cm

TABLE 3

Paper chromatography and electrophoresis: values of $R_{G\text{-}6\text{-}P}$ (movement relative to glucose 6-phosphate)

	Paper chromatography						High voltage electrophoresis [b]
Solvents [a]	A	B	C	D	E	F	
Migration time	7 h	15 h	2—5 day	2 day	2—3 day	1—2 day	pH 3·5 [c]
Compound							
Orthophosphate	1·16	0·80—0·95	6·4	1·2			1·54
Glucose 6-phosphate	1	1	1	1	1	1	1
Frucose 6-phosphate	1·1		1·3		1·3	1·7	
6-Phosphogluconate			1·25	0·74		1·6	
Arabinose 5-phosphate			1·65			1·35	
Ribose 5-phosphate	1·11	0·95—1·0	1·35—1·5	0·68		2·30	
Ribulose 5-phosphate	1·14	1	1·65—1·9			1·9	
Glucose	1·14	1·55	8·7—9	4·2		7	
Fructose			11			9·2	
Xylose			12·5				
Arabinose	1·5	1·58	11—13·5			10·5	
Ribose	1·5	1·62	14·5—16·5				
Erythrose			18·5				
Sorbitol			10			8·4	
Erythritol			16·5				
Gluconate	1·22	1·25	5·1—6·7			8·3	
Glucuronate		1·30	6·5				
Tartrate			16				
Glycerate			24·5				
Lactate		1·72	37				
Glycolate		1·59	31·5				

[a] Solvents: A = methanol–formic acid–water (16 : 3 : 1 v/v); B = n-propanol–ammonia–water (11 : 2 : 7); C = n-butanol–acetic acid–water (4 : 1 : 1); D = phenol saturated with water; E = t-butyl alchol–water–picric acid (80 ml : 20 ml : 2 g); F = ethyl acetate–acetic acid–water (9 : 2 : 2). [b] 40—60 V cm^{-1}. [c] Buffer pyridine–acetic acid–water (6·6 : 66 : 3000).

TABLE 4

Yields of the products of photolysis of [^{14}C]glucose 6-phosphate after radiochromatography. Initial concentration of glucose 6-phosphate 0·01M, illumination time 4 h

Solvent	$R_{G\text{-}6\text{-}P}$	Probable product	$h\nu$–N_2 Yield (%)	$h\nu$–O_2 Yield (%)
C	1	G-6-P	60	65
	1·3—1·7	?	4	2
	5·8—6			0·5
	6·4	HPO_4^{2-}		
	8·7		Trace	1
E	0·75		22	21·6
	0·92	}		
	1	G-6-P}	49	57
	(1·2)	(F-6-P) *	1·4	1·9
C After alkaline phosphatase	1	G-6-P	9·9—11·4	5·6—11
	1·8—2		4·7—5·6	3·6—6
	5·3		12·1—13·1	
	5·8		11·5—12·7	4·3—7·4
		Gluconate		5·8—8
	8·7	Glucose	31—33	52—70
	10·8	Arabinose	2·1—3	6·6—10·2
	13·5—14	Ribose(?)	0·35—0·5	1—4·5

* Fructose 6-phosphate used as carrier.

TABLE 5

Major ion fragments in the mass spectra of gas chromatographic peaks of photolysis products (converted to trimethylsilyl ether) of glucose 6-phosphate under oxygen

	Product		
m/e	Glucose 6-phosphate	6-Phospho-gluconate	Arabinose 5-phosphate
73	195	360	212
75	31	60	27
101		20	
103	10		8
116		16	
129	43	16	22
131		10	
133	12	24	15
147	41	84	52
157		13	
169			8
191	17	8	22
204	100	12	10
211	7	11	17
215			43
217	26	28	29
227			4
230			43
243	6		11
247		12	
259			3
271	9		
299	46	100	87
314		12	
315	16	38	100
328			15
333		18	
343			3
345	5		
357	11	26	
369			3
370	6		
371			3
373			2
387	46	44	2
445—448	0·9		t
485			2
575			6
672—677	0·7		
765		2	

t = Trace.

photolysis of glucose 6-phosphate under nitrogen and oxygen, respectively, is presented in Figure 3. As we had noted before, in oxygenated solutions, the formation of carboxylic acids is the major reaction.

Chromatographic Identification.—On the basis of paper chromatography and electrophoresis, as well as of g.l.c.–mass spectrometry of the trimethylsilyl derivatives, the products of photolysis in oxygenated solutions were identified as 6-phosphogluconate, arabinose 5-phosphate, phosphoglycerate, and phosphoglycolate. After photolysis under nitrogen, only arabinose 5-phosphate was identified.

Photolysis of 6-Phosphogluconate.—The oxidation of glucose 6-phosphate produces 6-phosphogluconate. This reaction may therefore be expected during the photolysis of glucose 6-phosphate under oxygen. 6-Phosphogluconate may undergo further photochemical degradations, and the secondary products of these reactions may appear also during the photolysis of glucose 6-phosphate. In order to test this, the photolysis of trisodium 6-phosphogluconate

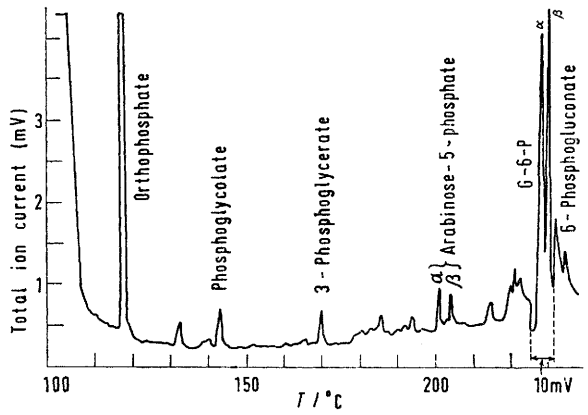


FIGURE 4 G.l.c. of trimethylsilyl derivatives of the products of photolysis of glucose 6-phosphate (0·01M) under oxygen for 2·5 h

solutions under oxygen was investigated. A comparative study of the products from glucose 6-phosphate and from 6-phosphogluconate, respectively, by paper chromatography (before and after hydrolysis by alkaline phosphatase) indicated that arabinose phosphate must be a major photolysis product from both sugar phosphates. Tentatively, we can, therefore, conclude that 6-phosphogluconate may be an intermediate in the photolysis of oxygenated solutions of glucose 6-phosphate to form arabinose 5-phosphate.

DISCUSSION

The photolysis of glucose 6-phosphate in aqueous solution may involve release of orthophosphate, dehydrogenation and other hydrogen transfer reactions, and carbon–carbon bond breakage, either as primary or as secondary steps. The extent of participation of such processes may be estimated in part on the basis of our experimental results, the formation of 6-phosphogluconate and arabinose 5-phosphate as predominant reaction products, as well as further degradation to three-, two-, and one-carbon fragments.

Release of Orthophosphate.—Orthophosphate elimination, together with dehydration, had been observed to be a predominant process in the photolysis of ethyl dihydrogen phosphate [4] and of the glycerophosphates,[5]

resulting in the formation of acetaldehyde and of glyceraldehyde (or dihydroxyacetone), respectively. By analogy, in the photolysis of glucose 6-phosphate, we would expect the formation of *gluco*-hexodialdose, CHO-$[CHOH]_4CHO$.

Such an oxidative dephosphorylation had indeed been observed in the radiolysis of glucose 6-phosphate by ionizing radiation, in which *gluco*-hexodialdose was observed as a major product.[7] One of the initial steps of this reaction was shown by e.s.r. on various alkyl dihydrogen phosphates, to be abstraction of a hydrogen atom, with formation of a radical ion [reaction (1)].[19] A similar

$$RCH_2OPO_3^{2-} \longrightarrow R\dot{C}HOPO_3^{2-} \qquad (1)$$

radical ion may also be involved in the photolysis of disodium glucose 6-phosphate, by hydrogen abstraction from either CH_2 or one of the CH groups.

Phosphate elimination, not involving dehydrogenation, may result in the formation of an anhydro-sugar. Such a process had been observed in the alkaline degradation of glucose 6-phosphate, in which 1,6-anhydroglucopyranose was detected in small yield.[8b]

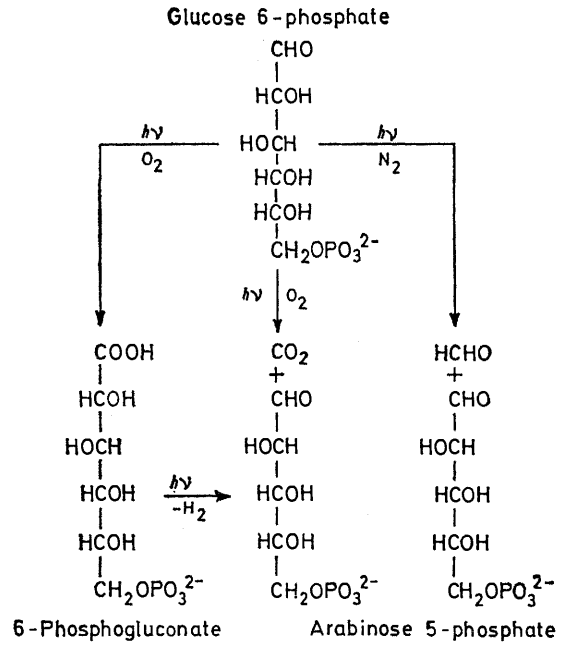
In the photolysis of glucose 6-phosphate, these simple phosphate elimination reactions seem not to occur in significant yields, as we failed to detect the formation of either *gluco*-hexodialdose or 1,6-anhydroglucopyranose. During photolysis under oxygen, we found that the rate of release of orthophosphate is slower than that of the disappearance of glucose 6-phosphate; this fact is in agreement with the preceding observations and it seems that release of orthophosphate is due to secondary processes of degradation. This is in contrast with the photolysis of other organic dihydrogen phosphates.[1,4,5]

Dehydrogenation and Other Hydrogen Transfer Reactions.—Oxidation of D-glucose by photolysis under oxygen leads to the formation of carboxylic acids, such as gluconic acid.[10,11] An analogous reaction is an important process in the photolysis of glucose 6-phosphate under oxygen, leading to 6-phosphogluconic acid. This photochemical dehydrogenation may be considered an abiological model of the important enzymic dehydrogenations of glucose 6-phosphate (with glucose 6-phosphate dehydrogenase and NADP), leading to 6-phosphogluconolactone and -gluconic acid, which are key steps in the biosynthesis of pentose sugars. Further steps of dehydrogenation must be involved in the degradation leading to phosphoglycerate and phosphoglycolate.

Carbon–Carbon Bond Breakage.—Breakage of the carbon chain between carbon atoms 1 and 2 has been reported for the photolysis of D-glucose, leading to arabinose as one of the major products.[10,11] The analogous reaction is observed in the present work. Photolysis of glucose 6-phosphate, both under nitrogen and under oxygen, leads to the formation of arabinose 5-phosphate. Under oxygen, phosphogluconate could be an intermediate in this reaction (see Scheme).

During radiolysis of glucose, breakage of the sugar skeleton between carbon atoms 2 and 3 as well as 3 and 4 was also observed.[10,11] In the case of glucose 6-phosphate, the analogous reactions lead to the formation of triose phosphates, such as glyceraldehyde 3-phosphate (or dihydroxyacetone phosphate), tetrose phosphates such as erythrose 4-phosphate, and under oxidizing conditions to acids such as 3-phosphoglycerate and glyceric and glycolic acids.

The observed formation of an acid-labile phosphate ester after photolysis of glucose 6-phosphate under oxidizing conditions ($t_{\frac{1}{2}}$ 30 min in N-H_2SO_4 at 100°) is in agreement with the Scheme, which is further supported by the identification of phosphoglycolate and phosphoglycerate (by g.l.c.–mass spectrometry).



SCHEME

The u.v. absorbing substances formed from sugars under a variety of conditions have been the subject of considerable discussion. In the radiolysis of D-glucose and of glucose 6-phosphate, various deoxy-sugar derivatives have been identified as products, and have been tentatively attributed as the cause of the u.v. absorption at *ca.* 275 nm.[7] Predominant formation of a simple degradation product, *e.g.* malonaldehyde, has also been suggested.[7] Another hypothesis is formation of an enone chromophore which is in tautomeric equilibrium with a dicarbonyl compound.[10c,11c] The primary process would thus be excitation of the ' acetal chromophore ' at C(1) of the glucose residue,[10c] possibly by an $n \longrightarrow \sigma^*$ transition of the non-bonding orbital on the oxygen atom of the pyranose ring. A similar compound may be formed in the photolysis of glucose 6-phosphate, particularly under nitrogen, in which the formation of an intense absorption band at 270 nm is observed.

The photolysis of D-glucose 6-phosphate in aqueous

[19] A. Begum, S. Subramian, and M. C. R. Symons, *J. Chem. Soc.* (*A*), 1970, 1334; C. M. L. Kerr, K. Webster, and F. Williams *J. Phys. Chem.*, 1972, **76**, 2846; A. Samuni and P. Neta, *ibid.*, 1973, **77**, 2425.

solutions is in many respects similar to that of D-glucose, in that the initial photochemical step occurs at least partly at C(1) to form the corresponding phosphogluconic acid, and from this by decarboxylation, D-arabinose 5-phosphate. This photolysis is in contrast to those of other alkyl phosphates which involve as primary step the formation of a radical ion, $R\dot{C}HOPO_3^{2-}$, followed by release of orthophosphate.[1,4,5] As proposed for the photolysis of glucose,[10] the stepwise photolytic decarboxylation of sugar phosphates may be considered a non-biological reversal of the photosynthesis of carbohydrate in living plant cells.

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